

Chapter 20

Colorimeters

GMDN Code	36910	38837	15146
ECRI Code	18-257	18-258	15-146
Denomination	Photometer, filter, automated	Photometer, filter, manual	Haemoglobin analysers (Haemoglobinometer)

PHOTOGRAPH OF COLORIMETER

Portable haemoglobinometer



Photo courtesy of Hemocue AB

PURPOSE OF THE COLORIMETER

A colorimeter is an electrically powered instrument which measures the concentration of analytes in coloured solutions. It is a simple version of a photometer. The difference in the quality of its filters makes it less sensitive. The colorimeter is used for clinical chemistry, namely for determining haemoglobin concentrations. Colorimeters are made by several manufacturers and include types with inbuilt individual removable filters or filter wheels for up to ten wavelengths. Some models are adapted for hot and humid climates with gelatine filters encased in glass to prevent fungal growth and coated individual components to prevent corrosion. Colorimeters may be

manual or semi-automated. Absorbance readings are done with needle or digital readouts. The haemoglobinometer is a portable colorimeter designed to provide direct, accurate haemoglobin concentration readings in g/dl or g/l. It will also be covered in this chapter.

OPERATING PRINCIPLE

A colorimeter uses filters to produce light of a single wavelength selected according to the colour of the solution being measured. The coloured light passes through the sample and the amount of light emerging is measured on a scale of absorbance. The absorbance is directly proportional to the concentration of the coloured compound in the solution according to Beer-Lambert law (see Chapter 11). It can usually measure reliably between 0 and 0.7 absorbance units. Calibration factors are higher for colorimeters than for photometers as they are less sensitive. Calibration factors for specific methods or reagents are usually provided by manufacturers or in the literature.

Haemoglobinometers measure the concentration of haemoglobin in blood. The majority of models is manually operated and uses main or battery cell power. New models have rechargeable batteries and/or use solar energy as a source of power. Most require dilution of blood before haemoglobin measurement. Some models use a device for collecting blood without dilution; these devices are single use and disposable, thus increasing the cost of haemoglobin estimation.

COMPONENTS

The basic components of colorimeters are similar to those of a photometer as shown in Figure 62, Chapter 19. As mentioned earlier in this chapter, these instruments are simpler and due to the quality of their filters, less sensitive. The light source may be a diode lamp emitting monochromatic light. Alternatively light produced by a tungsten or halogen lamp may be filtered to achieve the required wavelength. Depending on the model, the controls of the instrument may feature the following:

1. Display window
2. ON/OFF button
3. Cuvette chamber
4. Test button
5. Reference button
6. Various modes selection button, e.g., Absorbance/ %Transmittance, Kinetics (not on all models)

INSTALLATION REQUIREMENTS

1. A clean, dust, fume and smoke free environment, away from direct sunlight is required.
2. Unpack carefully and assemble following instructions from the manufacturer if applicable.
3. Place the instrument on a firm bench and, if required, near (no more than 1.5 m away) an electric power outlet with a ground pole.
 - a. The outlet must have its respective ground pole in order to guarantee the protection and safety

of the operator and the equipment. Colorimeters generally operate at 110-120 V/60 Hz or 220-230 V/50Hz.

- b. If not battery operated, protect the instrument from power surges using a voltage stabilizer.
4. Follow the manufacturer specifications for the installation of specific models.
5. For added safety, the instrument may be locked in a cupboard when not in use. This may not be possible for large models, although these could be locked in another fashion if judged necessary.

OPERATION OF THE COLORIMETER

Only staff trained and authorized to use the **colorimeter** are allowed to operate the instrument. This section is based on the use of the portable colorimeter model, equipped with inbuilt filters and a digital display. Other models may require different procedures and manufacturer's instructions should always be followed.

1. Connect the unit to the power supply and switch ON.
2. Allow 15 minutes for the instrument's optical and electronic systems to warm up.
3. Select the correct wavelength for the compound to be tested e.g. 540 nm for haemoglobincyanide.
4. Select "absorbance" using the Mode button.
5. Arrange all the required solutions in a test rack: blank (reagent containing no sample); standard of known concentration and test solutions (samples).

Figure 63. Controls on a portable colorimeter

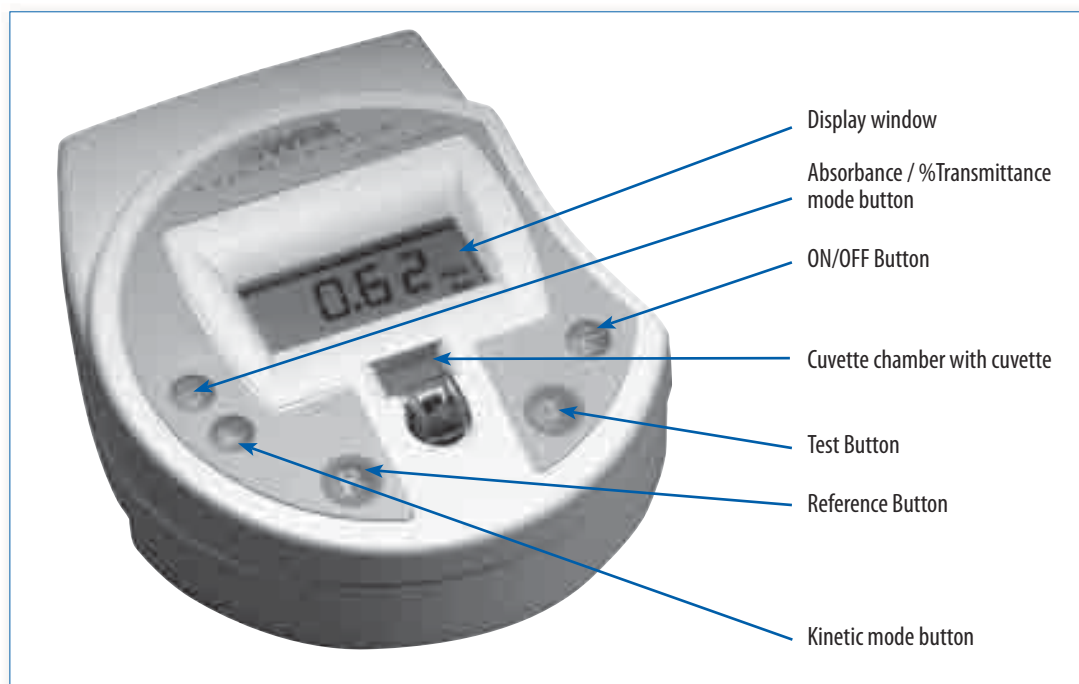


Photo courtesy of Biochrome Ltd

6. Carefully clean the cuvette using lint-free soft tissue or lens paper to avoid scratches. Always hold by the opaque ground side.
7. Transfer the blank solution into the cuvette and place it into the sample compartment with the clear sides facing the light path.
8. Close the chamber and set the display to zero using the SET BLANK control.
9. Remove the cuvette from the compartment and pour the solution back into its original test tube.
10. Pour the standard solution into the cuvette and read the absorbance.
11. Repeat step 9.
12. Read the test solutions in the same fashion.
13. Using a table of values obtained from a calibration curve derived from the instrument, read the concentration of the test samples against the absorbance.
14. After use, switch off the power supply and cover the equipment to protect it from dust.
15. Rinse the cuvette with distilled water, drain dry and wrap in soft material. Store carefully into a small box to prevent scratches and dust.
11. Remove the blank from the compartment and pour it back into the original test tube.
12. Pour the standard solution into the cuvette and place it in the compartment.
13. Close the cover and wait 3 sec. Register the reading from the digital display.
14. Remove the standard from the compartment and pour it back into the original test tube.
15. Pour the diluted sample solution into the cuvette and place it in the compartment.
16. Close the cover and wait 3 seconds and register the reading from the digital display.
17. Remove the sample from the compartment and pour it back into the original test tube.
18. Repeat steps 16-17 for each sample to be tested.
19. Rinse the cuvette with distilled water. Drain dry, wrap in soft material and store in a small box to prevent scratches.
20. Turn off by switching off or disconnecting at the wall socket if applicable. If not, remove the plug or disconnect the battery terminals.
21. Store in a locked drawer or in another suitable location.

OPERATION OF THE HAEMOGLOBINOMETER

Only staff trained and authorized to use the **haemoglobinometer** are allowed to operate the instrument. This section describes the operation of a portable haemoglobinometer with LED light source and digital display. Different models require different procedures and manufacturer's instructions should always be followed.

1. Connect the instrument to the power supply and switch ON or use the internal power source.
2. Place the ON/OFF switch on the ON position.
3. Choose readout to be used routinely, e.g. g/DL.
4. Warm-up time should be displayed in seconds if applicable. For other models wait 15 minutes or the time recommended by the manufacturer.
5. Prepare all the solutions in test tubes in a rack, i.e. blank, standards, test solutions.
6. Leave at room temperature for 10 minutes to equilibrate.
7. Meanwhile, carefully clean the cuvette using a soft tissue to avoid scratching.
8. Avoid touching the sides of the cuvette facing the light path; hold the cuvette by the opaque sides that will not face the light path.
9. Transfer the blank solution into the cuvette and place it in the sample compartment with the clear sides facing the light path.
10. Blank the instrument: close the cover and wait approximately 3 sec and adjust the display knob at 0:00.

ROUTINE MAINTENANCE

Maintenance should be performed by qualified personnel. This section describes general routine maintenance for colorimeters and haemoglobinometers. Some models may require different procedures. Always carefully follow the manufacturer's instructions for regular servicing and maintenance of the colorimeter or haemoglobinometer.

Frequency: Daily

1. Any spill on, or around the instrument should be cleaned immediately.
2. At the end of the day, turn off the instrument or disconnect the power source or the battery terminals as appropriate.
3. Keep the cuvette chamber empty and closed when not in use.
4. Cover the instrument after use. Store appropriately, protected from dust.

Frequency: As needed

1. Replace blown fuses and bulbs according to the manufacturer's instructions.
2. If the equipment is faulty, consult a qualified biomedical engineer.

Frequency: Monthly

The window and/or front surface of the photodetector should be inspected and cleaned with lens tissue.

Frequency: Every six months

1. Inspect the instrument visually to verify the integrity of its components according to the manufacturer's specifications.
2. Verify that the buttons or control switches and mechanical closures are mounted firmly and that their labels are clear.
3. Ensure that all the accessories are clean and intact.
4. Check the adjustment and condition of nuts, bolts and screws.
5. Make sure the electrical connections do not have cracks or ruptures. Test that these are joined correctly.
6. If applicable:
 - a. Verify that cables securing devices and terminals are free from dust, grime or corrosion.
 - b. Verify that cables are not showing signs of splicing or of being worn out.
 - c. Examine that the grounding system (internal and external) is meeting the electric code requirements.
7. Make sure the circuit switches or interrupters, fuse box and indicators are free from dust, corrosion and grime.
8. Check lamp alignment if recommended by the manufacturer.

Frequency: Annually

These tests must be performed by an electrician or engineer and results must be recorded and archived for follow-up through time.

1. Check the installation location for safety of the electrical and the physical infrastructures.
2. For instruments using main power:
 - a. Check that the voltage is appropriate and does not vary more than 5% from the voltage in the equipment specifications.
 - b. The polarity of the outlet is correct.
3. Check that there is sufficient space around the instrument for the connecting cables and for adequate ventilation.
4. Test the integrity of the counter and its cleanliness.
5. Verify that the instrument is away from equipment generating vibrations and direct solar radiation.
6. Check that there is no excessive humidity, dust or high temperature.
7. Ensure that there is no source of smoke, gas or corrosive emissions nearby.

General maintenance

Refer to the general maintenance of spectrophotometer in Chapter 11 for the cleaning of spills and replacement of batteries.

Cuvette use and maintenance

Cuvettes must be rigorously clean for accurate measurements. Clean these as described in Chapter 11. Additional recommendations are as follow:

1. Always hold cuvettes by their opaque, non-optical walls.
2. Unless specified by the operator's manual, do not perform any measurements without performing a blank determination.
3. Use a single cuvette or a set of matched cuvettes for proper performance of the instrument. Note: Absorbance of cuvettes should not exceed 0.01 when measuring distilled water. To avoid incorrect results, a cuvette exceeding this limit should not be used as part of a set unless it is matched with one with the same absorbance reading when measuring distilled water.
4. Remove bubbles present in the solution by gently tapping the cuvette with the finger.
5. Ensure that there is a high enough level of solution in the cuvette (above the light beam) so that the reflection of light from the surface does not interfere with the reading.
6. All solutions used and the specimen to be measured should be clear. If the mixed reagent solution and specimen is turbid, the measurement must be repeated after checking and confirming the cuvette's transparency and cleanliness.
7. If a kinetic measurement is performed over a long period of time, seal the cuvette to avoid evaporation causing erroneously high readings.
8. When performing readings on a series of specimens, readjust the zero every 5 to 10 measurements by reading the blank solution to avoid a drift of the zero.
9. Do not leave the cuvette in the instrument.
10. If using semi-micro or micro-cuvettes, ensure correct positioning in the light path to avoid false readings due to partially reflected light.
11. Store in a dust-free box to prevent damage as scratched or damaged cuvettes can lead to incorrect measurements.

Optical filters use and maintenance

1. Handle removable filters by the circumference to avoid contamination.
2. Keep spare filters in a dust-free box to insure protection from breakage or scratches.
3. Ensure that a filter is in its slot when the lamp is turned ON to avoid damage to the photocell. Store filters in the appropriate storage box when the instrument is not in use.
4. When the instrument is cool and turned OFF, clean the filters and optical window with lens tissue as instructed by the manufacturer.

Light source use and maintenance

1. Turn OFF the lamp after each use to maximize its life span. Some manufacturers recommend keeping a record log of the instrument lamp use.
2. Check lamp periodically. Replace if it is the cause of instability in the absorption signal.

Lamp alignment

The following are procedures to align new lamps. Refer to the instructions from the manufacturer to insure the procedure is performed according to specifications of the instrument model in use.

Realign the new lamp as follows:

1. Place a clean cuvette filled with distilled water in position in the instrument.
2. Set the meter to a mid-scale reading, e.g. at 50% transmission.
3. Move each optical component slightly in turn and check if the reading was affected.
4. If needed, adjust the lamp alignment for maximum transmission.
5. Alternatively, place a white card in front of the photocell (some instruments will allow this). Observe the image of the lamp on the card. It should be vertical and in focus. If not, adjust the lamp alignment until the best image is obtained.

Troubleshooting tables containing problems sometimes encountered with colorimeters are presented below. Since instrument models vary widely the following guidelines take precedence:

1. Always refer to the instruction manual from the manufacturer.
2. If an instrument fails to switch on, if applicable, check the electric socket outlet. Plug and check the fuse or the battery terminals.
3. In case of a major breakdown, consult a qualified biomedical engineer.

TROUBLESHOOTING TABLE

Automated Colorimeter

PROBLEM	PROBABLE CAUSE	SOLUTION
The colorimeter does not start.	The on/off switch is in the off position.	Move the switch to the on position.
	There is no electric energy in the feed outlet.	Verify the main electric feed. Verify that some electrical safety mechanism has not been misfired.
	The electric feed cable is not well connected.	Connect the feed cable firmly.
The keyboard or buttons do not respond.	The initialization of the equipment during start-up is incomplete.	Turn off the equipment and switch on again.
	An incorrect command was activated, during start-up.	
The serial port does not respond.	There was incomplete initialization of the equipment during start-up.	Turn off the equipment and switch on again.
	The interconnection cable is not connected well.	Verify the connection.
The LCD screen is difficult to read.	The contrast control is maladjusted.	Adjust the contrasts.
	Base lighting system burnt out.	Call the representative.
The printer is blocked.	Paper jam.	Remove the excess paper with finely pointed tweezers.
		Remove the paper and reinstall again.
The printer's paper does not auto feed or advance.	The printer paper is installed incorrectly.	Reinsert the roll of paper.
	The front edge of the paper is not aligned or folded.	Reinsert the roll of paper. Cut the front edge and realign in the feed system.
	The paper feed control does not respond.	Call the representative.
The cuvette does not enter in the sample holder compartment.	The cuvette is of wrong size.	Use the size of cuvette specified by the manufacturer.
	The cuvette's adjustment mechanism is incorrectly placed.	Correct the position of the adjustment mechanism.
The reading shows fluctuations.	There are interferences in the light's path.	Verify that the cuvette is not scratched.
		Verify that there are no particles floating in the cuvette.
		Rub the optic walls of the cuvette with a piece of clean cloth.
		Verify that the working range (wavelength and dilution) selected is appropriate for the sample analyzed.
The reading shows negative values. There is no absorbance reading.	There is no sample.	Add a sample to the solution.
	The cuvette is incorrectly positioned.	Verify the orientation of the cuvette. Clear sides should face the light path.
	The wavelength is erroneously selected.	Adjust the wavelength to the range compatible with the analysis.
	The equipment was calibrated with a sample in place of a standard solution.	Calibrate with a standard solution or with distilled water.

Non-automated Colorimeter		
PROBLEM	PROBABLE CAUSE	SOLUTION
The source lamp does not light up.	The filament is broken.	Replace the lamp.
	The safety fuse is burnt out.	Replace the fuse.
	There is resistance in the lamp's filament.	Replace the lamp.
	The voltage is incorrect.	Review the voltage. Check the feed source.
Low readings in the meter or in the galvanometer.	The source lamp is defective.	Replace the lamp.
	The photocell is dirty or defective.	Clean or replace the photocell.
	The multiplier is defective.	Change or repair the multiplier.
	The source lamp's voltage is low.	Adjust the voltage.

BASIC DEFINITIONS

Since these instruments are based on the photometry principles, relevant definitions may be found in Chapter 11.